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(Received 17 December 2008; published 29 May 2009)

The Michaelis-Menten (MM) equation is the basic equation of enzyme kinetics; it is also a basic building block of many models of biological systems. We build a stochastic and microscopic model of enzyme kinetics inside a small subcellular compartment. Using both theory and simulations, we show that intrinsic noise induces a breakdown of the MM equation even if steady-state metabolic conditions are enforced. In particular, we show that (i) given a reaction velocity, deterministic rate equations can severely underestimate steady-state intracellular substrate concentrations and (ii) different reaction schemes which on a macroscopic level are indistinguishable because they are described by the same MM equation obey distinctly different equations in subcellular compartments.

DOI: 10.1103/PhysRevLett.102.218103 PACS numbers: 87.16.A−, 82.20.Uv, 82.39.Fk

The Michaelis-Menten (MM) equation has been a cornerstone of enzyme kinetics for almost a century [1]. Its popularity stems from the fact that it provides a simple and straightforward recipe for characterizing the kinetic properties of enzymes. Determination of these properties is useful, for example, in the comparison of wild-type and mutant enzyme samples, which can lead to insight into the nature of certain diseases and also for the accurate modeling and simulation of large metabolic networks inside cells. The simplest enzyme-catalyzed reaction can be represented as $S + E \underset{k_1}{\overset{k_0}{\rightleftharpoons}} C \xrightarrow{k_2} E + P$. Substrate molecules ($S$) reversibly bind to enzyme molecules (E) with rate constants k_0 (forward reaction) and k_1 (backward reaction) to form transitory enzyme-substrate complex molecules (C) which then decay with rate k_2 into enzyme and product molecules (P). The enzyme acts as a catalyst, effectively speeding up the reaction by orders of magnitude. In classical physical chemistry, chemical kinetics are described by rate equations based on the law of mass action; the solution of these ordinary differential equations (ODEs) for the above enzyme-assisted reaction in quasisteady-state conditions (i.e., when the complex concentration is approximately constant) leads to a relationship between the rate of product formation and the substrate concentration (the MM equation):

$$\frac{d[P]}{dt} = \frac{v_{\max}[S]}{K_M + [S]}, \tag{1}$$

where $v_{\max} = k_2[E_t]$ and $K_M = (k_1 + k_2)/k_0$ is the Michaelis-Menten constant. The square brackets denote concentrations; note that since enzymes can either be in free (E) or complexed states (C), it follows that $[E] + [C] = [E_t]$, where $[E_t]$ is a constant denoting the total concentration of enzyme. This is the case of a batch reaction, namely, one in which the system is instantaneously prepared with some initial amount of substrate at time $t = 0$ and the reaction proceeds after this event without substrate replenishment; in such conditions, quasi-steady-state conditions ensue if the initial amount of substrate is much larger than that of enzyme [2] and the MM equation approximates well the kinetics. Note that $[S]$ in this case is changing with time, albeit the decay is so slow that it can be considered constant. If one considers the reaction to occur under metabolic steady-state conditions, for example, due to constant substrate replenishment, then in the limit of long times, the MM equation becomes exact from the point of view of the classical rate equations. It is the latter case which is the focus of this article.

The MM relation is derived from deterministic ODEs which intrinsically assume that (i) the reaction volume is so large that the effects of noise due to molecular discreteness are negligible, (ii) the environment is well mixed via normal diffusion. Both of these assumptions are unlikely to be valid inside cells: (i) reactions typically occur in very small volumes and hence a low copy number of molecules are involved, (ii) high molecular crowding in the cytoplasm significantly hinders diffusion and makes active transport a more desirable mode of transport in a number of situations [3]. In this Letter, we relax the first assumption, namely, that of large reaction volumes and show that the MM relation breaks down even if perfect steady-state conditions are enforced at all times. It will furthermore be shown that different reaction schemes described by the same MM relation in the framework of ODEs obey different rate equations on a mesoscopic scale and are hence distinguishable.

We consider a model of enzyme kinetics inside a single subcellular compartment. The reaction scheme is generalized from the one considered in the introduction to: $\xrightarrow{k_{\text{in}}} A \underset{k_b}{\overset{k_f}{\rightleftharpoons}} S + E \underset{k_1}{\overset{k_0}{\rightleftharpoons}} C \xrightarrow{k_2} E + P$. Molecules of type A are continuously supplied at a rate k_{in} to the compartment. They reversibly change to a second species S which is the substrate which binds to the enzyme and leads to formation of product P. In the simplest possible scenario, A and S are isomers involved in a spontaneous isomerization reaction or in a catalyzed pseudo-first-order isomerization reaction. Isomerization via keto-enol tautomerization is very com-

mon in biochemistry, for example, featuring prominently in glycolysis [4]; the keto form is typically the one which is involved in enzyme reactions. Another possible physical interpretation is that A and S represent two different molecular species which interchange between each other via a combination of first-order and/or pseudo-first-order reactions. If we ignore the fact that subcellular compartments have very small volumes and instead assume a compartment volume which is large enough so that molecular discreteness can be ignored and furthermore assume well-mixed conditions then the kinetics are described by the conventional deterministic and macroscopic ODEs:

$$d[A]/dt = k_{\text{in}} - k_f[A] + k_b[S], \quad d[P]/dt = k_2[C] \tag{2}$$

$$d[S]/dt = -(k_b + k_0[E])[S] + k_f[A] + k_1[C], \tag{3}$$

$$d[C]/dt = k_0[E][S] - (k_1 + k_2)[C]. \tag{4}$$

If the rate of input is less than the rate at which substrate is maximally converted into product, i.e., $k_{\text{in}} \leq k_2[E_t]$, then the system achieves a steady state in the concentrations of substrate and enzyme. In this regime, it is easy to verify that the rate of product production is related to the steady-state substrate concentration by the MM equation, Eq. (1); the equation is exact since steady-state conditions are enforced. Note that this is independent of the rate constants k_b and k_f and hence it follows that at the macroscopic scale, given two reaction schemes with different values of k_b or k_f but with same $v_{\max}$, K_M and reaction velocity, one cannot distinguish between the two reactions from measurements of $[S]$; this property generally follows for any set of intermediate reactions preceding the bimolecular enzyme-substrate reaction and is not a special property of the reaction scheme under consideration. If we now relax the condition that the reaction volume is very large then due to the expected low copy number of molecules and the consequent noisy dynamics, the mathematical description necessarily becomes probabilistic and in terms of integer number of molecules. The state of the system at any time is now described by the joint probability distribution function π and the equation of motion governing the time evolution of π is frequently called a master equation (for an introduction, see [5]). For our set of reactions, the governing master equation is

$$\begin{aligned}\frac{d\pi}{dt} = {} & k_{\text{in}}\Omega(E_A^{-1} - 1)\pi + \frac{k_0}{\Omega}(E_S^{+1}E_C^{-1} - 1)n_S n_E \pi \\ & + k_b(E_S^{+1}E_A^{-1} - 1)n_S\pi + k_f(E_A^{+1}E_S^{-1} - 1)n_A\pi \\ & + k_1(E_C^{+1}E_S^{-1} - 1)n_C\pi + k_2(E_C^{+1}E_P^{-1} - 1)n_C\pi,\end{aligned} \tag{5}$$

where $\pi = \pi(n_A, n_S, n_E, n_C, n_P, t)$ is the joint probability distribution function describing the system at any point in time t, Ω is the volume of the compartment, and $E_X^{\pm 1}$ are the step operators defined by their action on a general function $g(n_X)$ as $E_X^{\pm 1} g(n_X) = g(n_X \pm 1)$. The quantities n_X denote the integer number of molecules of type X. The master equation is nonlinear and cannot be solved exactly but it is possible to systematically approximate it by using an expansion in powers of the inverse square root of the volume of the compartment [5]. The method is as follows. The stochastic quantity n_X/Ω fluctuates about the macroscopic concentrations $[X]$; furthermore these fluctuations are known to be of the order of the square root of the number of particles:

$$n_X = \Omega[X] + \Omega^{1/2}\epsilon_X, \tag{6}$$

where ϵ_X represents noise. Accordingly the joint distribution function and the operators can now be written as functions of ϵ_X: $\pi = \Pi(\epsilon_A, \epsilon_C, \epsilon_S, \epsilon_P, t)$ and $E_X^{\pm 1} = 1 \pm \Omega^{-1/2}\partial/\partial\epsilon_X + \frac{1}{2}\Omega^{-1}\partial^2/\partial\epsilon_X^2 + O(\Omega^{-3/2})$; using these new variables the master equation Eq. (5) takes the form

$$\begin{aligned}\frac{\partial\Pi}{\partial t} - \Omega^{1/2}\left(\frac{d[A]}{dt}\frac{\partial\Pi}{\partial\epsilon_A} + \frac{d[C]}{dt}\frac{\partial\Pi}{\partial\epsilon_C} + \frac{d[S]}{dt}\frac{\partial\Pi}{\partial\epsilon_S} + \frac{d[P]}{dt}\frac{\partial\Pi}{\partial\epsilon_P}\right) \\ = a_1\Omega^{1/2}\Pi + a_2\Omega^0\Pi + a_3\Omega^{-1/2}\Pi + O(\Omega^{-1}),\end{aligned} \tag{7}$$

where

$$\begin{aligned}a_1 = -\Big[& (k_{\text{in}} - k_f[A] + k_b[S])\frac{\partial}{\partial\epsilon_A} + k_2[C]\frac{\partial}{\partial\epsilon_P} + (k_0[E][S] \\ & - (k_1 + k_2)[C])\frac{\partial}{\partial\epsilon_C} + (k_f[A] + k_1[C] \\ & - (k_b + k_0[E])[S])\frac{\partial}{\partial\epsilon_S}\Big],\end{aligned} \tag{8}$$

$$\begin{aligned}a_2 = {} & \frac{1}{2}k_{\text{in}}\frac{\partial^2}{\partial\epsilon_A^2} + k_f\left[\frac{\partial}{\partial\epsilon_A} - \frac{\partial}{\partial\epsilon_S}\right]\epsilon_A + k_b\left[\frac{\partial}{\partial\epsilon_S} - \frac{\partial}{\partial\epsilon_A}\right]\epsilon_S \\ & + \frac{1}{2}\left(\frac{\partial}{\partial\epsilon_S} - \frac{\partial}{\partial\epsilon_A}\right)^2(k_f[A] + k_b[S]) + \frac{1}{2}\left(\frac{\partial}{\partial\epsilon_S} - \frac{\partial}{\partial\epsilon_C}\right)^2 \\ & \times (k_0[S][E] + k_1[C]) + k_1\left[\frac{\partial}{\partial\epsilon_C} - \frac{\partial}{\partial\epsilon_S}\right]\epsilon_C \\ & + k_2\left[\frac{\partial}{\partial\epsilon_C} - \frac{\partial}{\partial\epsilon_P}\right]\epsilon_C + \frac{1}{2}k_2\left(\frac{\partial}{\partial\epsilon_P} - \frac{\partial}{\partial\epsilon_C}\right)^2[C] \\ & + k_0\left[\frac{\partial}{\partial\epsilon_S} - \frac{\partial}{\partial\epsilon_C}\right](\epsilon_S[E] - \epsilon_C[S]),\end{aligned} \tag{9}$$

$$\begin{aligned}a_3 = {} & \frac{1}{2}\left(\frac{\partial}{\partial\epsilon_S} - \frac{\partial}{\partial\epsilon_C}\right)^2(k_0\epsilon_S[E] - k_0\epsilon_C[S] + k_1\epsilon_C) \\ & - k_0\left[\frac{\partial}{\partial\epsilon_S} - \frac{\partial}{\partial\epsilon_C}\right]\epsilon_S\epsilon_C + \frac{1}{2}k_2\left(\frac{\partial}{\partial\epsilon_P} - \frac{\partial}{\partial\epsilon_C}\right)^2\epsilon_C \\ & + \frac{1}{2}\left(\frac{\partial}{\partial\epsilon_S} - \frac{\partial}{\partial\epsilon_A}\right)^2(k_f\epsilon_A + k_b\epsilon_S).\end{aligned} \tag{10}$$

Note that ϵ_E does not feature in the expansion because it is not an independent variable but is equal to $-\epsilon_C$. Note also that in Eq. (10) terms which involve products of first and second-order derivatives, third-order derivatives, or higher have been omitted—these do not affect the low-order moment equations which we will be calculating. To

make Eq. (7) a proper expansion in powers of $\Omega^{-1/2}$, it is necessary to equate the terms proportional to $\Omega^{1/2}$ on both sides of the equation; this leads to the macroscopic equations, Eqs. (2)–(4). Note that this is an important benchmark, since it verifies that in the limit of very large volumes, the microscopic stochastic equation Eq. (5) leads to the correct macroscopic law that one would write down based on mass action. This leaves us with an equation of the form $\partial\Pi/\partial t = a_2\Pi + a_3\Omega^{-1/2}\Pi + O(\Omega^{-1})$; this equation contains information regarding the deviations from the macroscopic rate laws and is thus central to the topic of this Letter.

First we consider terms in the expansion to order Ω^0; the resulting equation is a multivariate Fokker-Planck equation. The nth moments of the noise $\langle\epsilon_X^n\rangle$ can be obtained by multiplying both sides of the equation by ϵ_X^n and integrating over all variables on which Π is dependent. The first moments at equilibrium take the trivial values $\langle\epsilon_A\rangle = \langle\epsilon_C\rangle = \langle\epsilon_S\rangle = 0$; from Eq. (6) it follows that to this order there are no new corrections to the values of the steady-state concentrations predicted by the macroscopic equations, i.e., $\langle n_X/\Omega\rangle = [X]$. The second moments are much more laborious to compute; they are given by a set of 6 coupled linear differential equations of the form $\frac{\partial}{dt}\mathbf{Z} = \mathbf{M}\cdot\mathbf{Z} + \mathbf{N}$. The column vectors $\mathbf{Z}$ and $\mathbf{N}$ are defined by $\mathbf{Z}^T = \langle[\epsilon_S^2, \epsilon_C^2, \epsilon_A^2, \epsilon_A\epsilon_S, \epsilon_A\epsilon_C, \epsilon_S\epsilon_C]\rangle$ and $\mathbf{N}^T = [(k_0[E] + k_b)[S] + k_1[C] + k_f[A], k_0(K_M[C] + [S][E]), k_{\text{in}} + k_f[A] + k_b[S], k_f[A] - k_b[S], 0, -k_0([S][E] + k_1'[C])]$. The nonzero entries of the 6×6 matrix $\mathbf{M}$ are the following: $M_{11} = -2k_0([E] + k_b')$, $M_{14} = 2k_f = -M_{33} = 2M_{43} = 2M_{65}$, $M_{16} = 2k_0(k_1' + [S]) = 2M_{45} = 2M_{62}$, $M_{22} = -2k_0([S] + K_M)$, $M_{26} = 2k_0[E] = 2M_{61}$, $M_{34} = 2k_b = 2M_{41}$, $M_{44} = -k_0(k_f' + k_b' + [E])$, $M_{66} = -k_0(k_b' + K_M + ([E] + [S]))$. The primed quantities indicate that they are divided by k_0. Note that these equations are independent of ϵ_p since the product is the end result of the reaction and hence can in no way influence the fluctuations in the number of molecules of types A, S, and C. This set of linear equations is exactly solvable though the algebra is tedious because of the high dimensionality of the system; we compute fluctuations in the limit $t\to\infty$ when all initial transients have decayed. Here we give only the expression for the correlator of the noise in the concentrations of complex and substrate, though the others can be computed in a similar fashion:

$$\langle\epsilon_S\epsilon_C\rangle = \frac{(1-\eta)^2 K_M[E_t](\alpha_1[E_t]\eta^2 + \alpha_3\eta + K_M k_f k_0)}{[E_t]\eta^2(\alpha_1[E_t]\eta^2 + \alpha_3\eta + K_M\alpha_{12}) + K_M\alpha_2^2\eta + k_0 K_M^2\alpha_2}, \tag{11}$$

where $\alpha_1 = k_0(k_2 + k_f)$, $\alpha_2 = k_b + k_f$, $\alpha_{12} = \alpha_1 + k_0\alpha_2$ and $\alpha_3 = k_f^2 + k_b\alpha_1$ and $\eta = 1 - k_{\text{in}}/v_{\max}$ (steady-state concentrations of substrate occur only over the range $0 \le \eta < 1$). Note that the correlator is evaluated using the steady-state concentrations given by the deterministic equations; this is since, as previously noted, to this order there are no corrections to the latter equations. Now we consider terms of order $\Omega^{-1/2}$ and ignore higher orders; these terms give information about how the equilibrium fluctuations are affected by (i) the nonlinear terms in the macroscopic equation (i.e., the bimolecular interaction of substrate and enzyme) and (ii) single-particle events, such as those occurring when the enzyme is close to saturation and there are few available free enzyme molecules for substrate binding [5]. Computing the first moments of the noise in the complex concentration gives

$$d\langle\epsilon_C\rangle/dt = -k_0([S] + K_M)\langle\epsilon_C\rangle + k_0[E]\langle\epsilon_S\rangle - k_0\Omega^{-1/2}\langle\epsilon_S\epsilon_C\rangle. \tag{12}$$

This equation of motion can also be obtained by a completely different approach. Arguing on purely physical grounds, one expects $d\langle n_C/\Omega\rangle/dt = \langle f(n_C/\Omega, n_S/\Omega)\rangle$ with the proviso that in the limit $\Omega\to\infty$, the function f becomes equal to the right-hand side (rhs) of Eq. (4). Taylor expanding $f(x + dx, y + dy)$ to second order with $x = [C]$, $y = [S]$ and $dx = \Omega^{-1/2}\epsilon_C$, $dy = \Omega^{-1/2}\epsilon_S$, taking the average over noise, and equating to $d\langle n_C/\Omega\rangle/dt$ one obtains exactly Eq. (12). Note that this alternative derivation relies solely on the macroscopic equations, though the evaluation of the cross correlator $\langle\epsilon_S\epsilon_C\rangle$ can only be obtained from the master equation. The steady-state condition, $d\langle n_P/\Omega\rangle/dt = k_{\text{in}} = k_2\langle n_C/\Omega\rangle$ and $k_{\text{in}} = k_2[C]$, implies that the average concentration of complex cannot possibly be affected by noise but it has to be equal to the macroscopic value (no such condition exists for the substrate concentration); from this it follows that $\langle\epsilon_C\rangle = 0$ to any order in the expansion for the master equation. Substituting the latter in Eq. (12) results in $\langle\epsilon_S\rangle > 0$, which implies

$$\Lambda = \frac{\langle n_S/\Omega\rangle}{[S]} = 1 + \frac{\langle\epsilon_S\epsilon_C\rangle}{K_M[E_t](1-\eta)\Omega}. \tag{13}$$

The correction factor on the rhs of this equation can be estimated using the value of $\langle\epsilon_S\epsilon_C\rangle$ given by the expansion to order Ω^0. Thus Eqs. (11) and (13) together approximate the deviations from the MM equation due to intrinsic noise. The deviations are in the range $1 < \Lambda < 1 + (\Omega K_M)^{-1}$. The expansion of the master equation is about the macroscopic concentrations and hence one expects Eq. (13) to hold when the 2nd term on the rhs is not very large. However, the alternative derivation of Eq. (12) (and also the simulations—see later) suggest the condition for its validity is not so restrictive. It is found that all higher-than-second-order terms in the alternative derivation yield no corrections to Eq. (12). This strongly suggests that the accuracy of Eq. (12) [and hence of Eq. (13)] is simply limited by the accuracy of the estimate of $\langle\epsilon_S\epsilon_C\rangle$. Note that the deviations from the MM equation are pronounced for

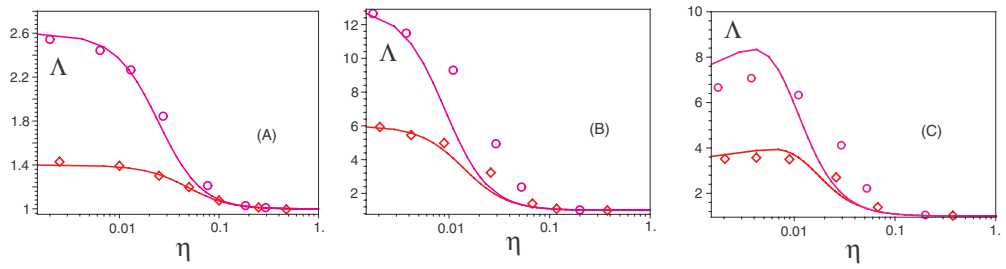

FIG. 1 (color online). Dependence of the deviations from the MM equation Λ on the parameter $\eta = 1 - k_{\rm in}/v_{\rm max}$ for relatively large (A) and small $K_M\Omega$ (B),(C) for a system with 1000 enzyme molecules in a compartment of unit volume. The solid lines show the theoretical predictions [Eq. (13) together with Eq. (11)] while the data points are those obtained from simulation. The parameters are as follows: $K_M = 5/2$ (red or gray diamonds) and $K_M = 5/8$ (magenta or light gray circles) in (A); $K_M = 1/5$ (red or gray diamonds) and $K_M = 1/12$ (magenta or light gray circles) in (B). In (A) and (B), the backward reaction $S \rightarrow A$ does not occur: $k_f = 1$, $k_b = 0$. (C) has the same parameters as (B) but now both backward and forward reactions involving A occur: $k_f = k_b = 1$. In all cases, $k_1 = 1$, $k_2 = 4$; K_M is varied through k_0.

small values of K_M, in which case the bottleneck in the catalytic process is the decay of a complex rather than an enzyme-substrate combination, leading to correlations between successive binding events. Simulations of the reaction scheme were carried out using Gillespie's exact stochastic simulation algorithm, conveniently implemented in the standard software, Dizzy, to test the accuracy of our predictions [6]. For a given fixed set of rate constants and total number of enzyme molecules, the rate of particle input into the compartment $k_{\rm in}$ was varied incrementally from 0 to $v_{\rm max}$; for each different value of $k_{\rm in}$, the number of substrate particles n_S was measured in the limit of long times. Ensemble averaging the latter over a number of independent simulations yielded $\langle n_S \rangle$ from which one can calculate Λ. Figures 1(a) and 1(b) show the results of simulations testing the dependence of the corrections to the MM equation on K_M and η for the case in which the backward reaction $S \rightarrow A$ is not allowed, i.e., $k_b = 0$. The predictions are in very good quantitative agreement with the numerics over the whole range of η for large $K_M\Omega$ [Fig. 1(a)] but underestimate the simulation results for intermediate values of η if $K_M\Omega$ is relatively small [Fig. 1(b)]; the latter is to be expected since as $K_M\Omega \rightarrow 0$, the fluctuations become very large and necessarily the expansion's predictions would not be expected to be highly accurate. However, the good match at small η is surprising and does suggest that terms beyond $\Omega^{-1/2}$ in the master equation expansion have little effect on the corrections to the MM equation [this is also suggested by the alternative derivation of Eq. (12), as previously mentioned]. In Fig. 1(c) both forward and backward reactions of A interconverting into S are allowed; given the choice of parameters [see Fig. 1 caption], the macroscopic equations would predict that this case should be no different than that of Fig. 1(b). However, this is not the case; because the stochastic corrections to the MM equation depend on k_f and k_b, it follows that at the mesoscopic scale, for a given rate of product formation (as given by η), the substrate concentration depends on the details of any reactions preceding the enzyme-substrate reaction. These corrections are of relevance only when η is small, i.e., when the enzyme is nearly saturated with substrate.

The mesoscopic theory developed in this Letter interpolates between the classical case of many enzyme molecules in a macroscopic volume (described by the MM equation) and the microscopic case of a single enzyme in a small volume (for example, see [7], which also predicts a breakdown of the MM equation for a single-enzyme system) thus spanning a wide range of biologically relevant scales. Since the range of K_M is approximately 1–5000 μM [8] and compartments are typically larger than 50 nm diameter (vesicles being at this lower end limit [3]), it follows that the amplification factor Λ can take values as high as $\sim$25 inside cells. This upper limit is achieved when the enzymes operate near saturation. Besides the clear breakdown of the MM equation in such cases, this also suggests that for given rates of product formation, there can be significant discrepancies between the steady-state intracellular metabolite concentrations and those measured in macroscopic volumes.

The author gratefully acknowledges support from SULSA (Scottish Universities Life Sciences Alliance).

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